REMARKS

Claims 1-18 are pending in the present application. Reconsideration on the merits is respectfully requested in light of the following remarks.

Rejections under 35 USC § 102

Claims 1-8 and 10-18 stand rejected under 35 U.S.C. § 102(b) as being anticipated by US Patent 5,981,165 to Weiss et al. (hereinafter "Weiss"). Applicants respectfully traverse.

The cells described as "embryonic stem cells" in Weiss are primary neural cells, as prepared in Example 3. In other words, the primary neural cells are already directed to ectodermal neural cells by the process of the development of an individual (stem cells prepared from embryo or neural stem cells from embryo).

On the other hand, the "embryonic stem cells" which are the subject of the present invention have an ability of differentiating into all of the cells, ectoderm, endoderm, or mesoderm. In addition, the embryonic stem cells are cells established as a cell line from an inner cell mass of blastocysts, so that they are clearly distinguishable from the primary neural cells in Weiss.

Therefore, Weiss using the cells directed to ectodermal neural cell, i.e. cells already destined to one direction, is essentially different from the present invention using cells capable of differentiating into all of the cells, ectoderm, endoderm, or mesoderm. Thus, the present invention cannot be anticipated from Weiss.

In addition, Weiss describes that the differentiation into DA-neurons occurs in the presence of FGF-2, as described in various parts of Weiss (Abstract; column 1, lines 44-50; column 2, lines 56-67; Example 2: column 12, lines 2-6; Example 8: column 15, lines 1-15; and lines 19-23). Weiss does not contain any description at all on the differentiation into DA-neurons under the conditions of "ACM," "ACM+EGF," "ECM," or "BCM+EGF."

On the other hand, in the present invention, the differentiation into neural cells occurs only with astrocyte-conditioned medium (ACM). Accordingly, the present invention cannot be anticipated from Weiss, within the meaning of 35 U.S.C. § 102(b). Reconsideration and withdrawal of the outstanding rejection are respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 1-3 and 10-12 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. (hereinafter "Zhang") in view of Flax et al. (hereinafter "Flax"). Applicants respectfully traverse.

Zhang reports that human embryonic stem (ES) cells cannot be maintained in an undifferentiated state in the absence of FGF-2 (Ref. 4 of Zhang). From this report, it is considered in Zhang that the differentiation occurs by removing FGF-2 and forming embryonic bodies (EB). Therefore, if FGF-2 is added, the differentiation would have been suppressed.

In the neural stem sphere used in the present invention, the suppression of differentiation into the neural stem cells (NSC) does not take place even when FGF-2 is added upon suspension culture in ACM. Thus, the present invention possesses a synergistic effect, contrary to that described in Zhang, which enhances the differentiation. The effect for FGF-2 upon the suspension culture as described above is reversed; therefore, it is clear that the present invention is completely different from that taught in Zhang.

The method of Zhang allows differentiation of the cells by an EB method to form neural tube-like structure on day 7 of the adhesion culture. By contrast, in the present invention, a sufficient amount of neural stem cells (NSC) are differentiated day 4 after the suspension, more in the suspension culture, not the adhesion culture. In the present invention, it is reasonable to consider that the reason that the NSC are differentiated only on a surface layer of the neural stem sphere is that a factor in ACM penetrates into the neural stem sphere, thereby enhancing the differentiation into NSC, proving that the ES cells are subjected to direct differentiation.

The NSC are reportedly neuroepithelial stem cells and radial glia of the ventricular zone in embryo, or astrocytes of the subventricular zone and subgranular zone in adult (*See* **Attachment 1: Doetsch, F., *Nature Neurosci. 6,* 1127-1134, 2003**). The fact that the NSC are differentiated in the order of neuroepithelial stem cells → radial glia → astrocytes, strongly suggests that the differentiation into astrocytes occurs in a default state of brain development.

Therefore, the fact that almost all of the ES-derived NSC are differentiated into astrocytes by removing FGF-2 agree with the phenomenon in the development of the brain in a living body.

Upon the differentiation into the neural cells in the brain, firstly the differentiation into neurons occurs, and subsequently the differentiation into astrocytes and oligodendrocytes occurs in accordance with the progress of the time axis (*See* **Attachment 2: Temple, *S., Nature* 414, 112-117, 2001**). The ES-derived NSC of the present invention are differentiated into astrocytes in a default state as mentioned above, while almost all of the cells are differentiated into neurons by providing an exogenic differentiation stimulation called ACM.

The NSC of Zhang are differentiated into three kinds of neural cells, namely neurons, astrocytes, and oligodendrites. Taking into consideration the axis of time from the development of the brain, it is understood that the neural stem cells of the present invention are more undifferentiated than those of Zhang, so that the cells of the present inventions seem to exhibit the nature close to that of the neuroepithelial stem cells.

In order that small elongated cells congregated in the center shown in Fig. 1-A of Zhang form a neural tube-like structure shown in Fig. 1-B with the time course, Zhang merely mentions an experimental tool to study human neural tube formation under controlled conditions (Zhang, p. 1131, second column, second paragraph), i.e. ES cell-derived neural precursor cells, recapitulate early steps of nervous system development in that neural tube-like structures are formed, merely stating that the process of development is reproduced. Therefore, Zhang does not directly relate to the differentiation of the ES cells into NSC.

Flat cells are migrated in the periphery of the adherent EBs; however, these cells are negative against markers for neurons, astrocytes, oligodendrites, and ES cells. Therefore, under the conditions of Zhang, many of ES cells are differentiated into unidentified cells; therefore, it is obvious that the ES cells cannot be directly differentiated into NSC. An advantage of the Zhang method is to collect the cells only having a rosette structure utilizing the difference in adhesion from unidentified flat cells, but never describing that Zhang performs direct differentiation of the ES cells.

A medium DMEM/F12 plus supplements used in Zhang is a general basic medium for preparing an astrocyte conditioned medium, and the medium is completely different from ACM containing various factors produced by astrocytes. As mentioned above, the method of Zhang merely prepares EB by removing FGF-2; therefore, Zhange does not render the present invention obvious.

Incidentally, the article contribution of Flax describes NSC collected from human fetal telencephalon that are cryopreserved, so that Flax is distinguishable from the teachings of the present invention in the cell species.

Rejections under 35 USC § 102/103

Claims 1-18 stand rejected under 35 U.S.C. §102(b) as anticipated by Zhang et al. or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Pataky et al. (hereinafter "Pataky"). Applicants respectfully traverse.

The comments regarding Zhang in the context of the discussion of the above 35 U.S.C. § 103(a) rejection are likewise applicable to the outstanding rejection.

Further, Pataky does not cure the deficiencies discussed with regard to Zhang. Pataky reports that neuronal axons of the CNS are regenerated in the spinal cord injuries (Refs. 65 and 66 of Pataky). While Pataky makes references to these publications, the main theme of the article

is to evaluate what sort of factors enhance survival in regenerable bulbospinal neurons against injuries caused by axotomy.

The effect of enhancing survival of ACM is such that astrocyte-conditioned medium also enhances the survival of bulbospinal neurons, supporting the hypothesis that non-neuronal cells are important mediators of trophic effects observed in vitro (page 366, second paragraph, last sentence of Pataky), to expect the enhancement of the survival by nonneuronal cells (including astrocytes) in the periphery of the injured spinal neurons.

The bulbospinal neurons prepared from E8 Embryo retrograde-labeled with DiI have already ended differentiating into neurons (within the developing chick brain stem, neurogenesis is complete prior to E5; page 367, first paragraph, first sentence of Pataky), so that outgrowth of neurites from bulbospinal neurons is caused by regeneration.

Therefore, Pataky which acknowledges that the differentiation into neurons is ended can no way expect the effect of nerve cell differentiation in ACM.

Accordingly, as described above, the present invention is not obvious over Pataky. Reconsideration and withdrawal thereof are respectfully requested.

In view of the foregoing, Applicants believe the pending application is in condition for allowance. A Notice of Allowance is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Monique T. Cole, Reg. No. 60,154 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.147; particularly, extension of time fees.

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Respectfully submitted,

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Attachments: Doetsch, F., *Nature Neurosci.*, Vol. 6, 1127-1134, 2003
Temple, *S.*, *Nature*, Vol. 414, 112-117, 2001